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SHORTER ARTICLES AND DISCUSSION

NOTES ON A DIFFERENTIAL MORTALITY OBSERVED BETWEEN TENEBRIO OBSCURIS AND T. MOLITOR

I RECENTLY had occasion to subject some meal worms for short periods of time to a temperature considerably higher than that of the laboratory. Although the experiment was begun with a different purpose in view, there has been one feature noted which seems worth recording at this time.

The meal worms used consisted of the larvæ of *Tenebrio molitor* Linn. and *T. obscuris* Fabr. These larvæ are very readily distinguished from each other by the fact that the pigment in the integuments of *T. molitor* is yellow to orange-brown, while that of *T. obscuris* is almost black. In other features the larvæ resemble each other to a remarkable extent.

EXPERIMENTS WITH ELEVATED TEMPERATURE

The worms were placed in large Stender dishes containing a little meal and the dishes were then placed in a large, constant temperature incubator, being insulated from the bottom of the incubator by a cork ring and care being taken that the glass sides of the dishes did not come in contact with the copper sides of the incubator. The temperature was frequently noted through the glass door of the incubator and was also recorded on a maximum and minimum thermometer placed inside of the incubator and likewise insulated from contact with the copper sides or floor.[1]

Three major experiments were made after a probable differential mortality had been observed. These experiments are summarized below.

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[1] A much higher temperature is obtained if contact with the walls or floor is allowed. When an experimental dish was not insulated from the floor all of the larvæ which it contained were dead when larvæ in an insulated dish were still active.

Experiment 1.—104 larvæ (53 *T. molitor* and 51 *T. obscuris*). Kept at a temperature of 43° C. for 3 hours. These were the survivors of a previous heating at 41°–42° for 4 hours in which no record was kept of the number dying. Count made 24 hours after last heating.

	T. molitor	*T. obscurus*
Normal or nearly normal	2	44
Sluggish	13	6
Barely alive	13	1
Dead	25	none
Per cent. dead	47.17	—

Experiment 2.—50 larvæ (25 *T. molitor* and 25 *T. obscuris*). Heated 3 hours at 43° C. Not previously heated. Count made 24 hours later

	T. molitor	*T. obscurus*
Normal or nearly normal	none	none
Sluggish	none	none
Barely alive	none	12
Dead	25	13
Per cent. dead	100	52

Experiment 3.—441 larvæ (204 *T. obscuris* and 237 *T. molitor*). Heated at 41.5°–42° for 3.5 hours.

	Count in 24 Hrs.		Count in 48 Hrs.		Count in 96 Hrs.	
	T. mol.	*T. obs.*	*T. mol.*	*T. obs.*	*T. mol.*	*T. obs.*
Normal or nearly normal	none	176	none	164	none	164
Sluggish	none	7	none	7	21	6
Barely alive	195	15	160	21	128	10
Dead	42	6	77	12	88	24
Per cent. dead	17.72	2.94	32.50	5.88	37.14	11.77
Per cent. barely alive	82.28	7.35	67.50	10.29	54.01	4.90

Inasmuch as these experiments show a remarkable differential mortality, two other series of experiments were undertaken, in one of which the external influence used was exposure to cold for a long period of time and in the other set, exposure to an atmosphere of pure carbon dioxide.

EXPERIMENTS WITH COLD

Two experiments were made on the influence of cold as affecting the death rate. Two large Stender dishes, each of which contained 50 *T. molitor* and 50 *T. obscuris,* were placed inside of a battery jar, together with a maximum and minimum thermometer, the top of the jar was closed with a sheet of rubberized cloth to keep out moisture, and the whole was then fastened outside a laboratory window, in such a manner that the bottom rested upon a concrete slab, but the remainder of the jar was not in contact with the laboratory walls. The experiment ran from December 28, 1912, to March 9, 1913.

Unfortunately for this experiment, the winter at Cold Spring Harbor was unusually mild and the minimum temperature recorded in the jar was only —10° C. with a maximum of +11°.

The jar was then taken into the laboratory and after standing at room temperature for 24 hours a count was made. A second count was made six days later with the results given below.

	First Count				Second Count	
	Experiment 1		Experiment 2		Exps. 1 and 2 Combined	
	T. obs.	*T. mol.*	*T. obs.*	*T. mol.*	*T. obs.*	*T. mol.*
Alive	40	47	33	44	50	91
Dead	10	3	17	6	50	9
Per cent. dead	20	6	34	12	50	9

EXPERIMENTS WITH CARBON DIOXIDE

In the third set of experiments, the influence of carbon dioxide on the differential mortality, approximately 75 larvæ of each species were placed in each of three gas wash bottles (E. & A. No. 3,658). The wash bottles were connected together with rubber tubing and carbon dioxide from a Kipp generator (limestone and hydrochloric acid) was slowly passed through the apparatus during the entire course of the experiment. The carbon dioxide was first washed through a saturated solution of sodium bicarbonate and then through distilled water before passing to the bottles containing the larvæ.[2]

The stream of carbon dioxide was started at 12 M., December 19. At 12:05 P.M. the jar nearest the generator (Jar No. 1) showed no movement of *T. molitor* but the *T. obscuris* were still very active; at 12:07 P.M. only a few *T. obscuris* were moving in jar No. 1 and nearly all of the *T. molitor* in jar No. 2 were dormant; at 12:09 all of the *T. molitor* in each of the three jars were in a state of "suspended animation" but a few *T. obscuris* were still moving in jar No. 1; 12:11 P.M. "some movement in each jar but only of *T. obscuris*"; 12:20 P.M. "There are still a few *T. obscuris* moving in each jar, these have bubbles at their mouth"; 12:25 "Still a slight movement of a few but the 'suspended animation' is practically complete"; 12:30 P.M. "No movement in any."

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[2] I have already shown (*J. Biol. Chem.*, **10**, p. 90, and AMER. NAT., **45**, pp. 749–750) that it is possible to keep insects in pure carbon dioxide for hours without causing death, although to all appearances they are dead within a very few minutes after being subjected to the action of the gas.

An analysis of the gas passing through the apparatus was made at 3 P.M., December 19, and it was found to consist of 98.92 per cent. by volume of carbon dioxide (absorbed by KOH) and 0.27 per cent. of oxygen (absorbed from the residual gas by alkaline pyrogallol). Another analysis was made at 11 A.M., December 20, showing 99.15 per cent. of carbon dioxide and 0.04 per cent. of oxygen.

At the end of each experiment the last wash bottle of the chain was detached from its mate, a rapid current of air was drawn through it for several minutes, and then the larvæ were shaken out into a large open dish and allowed to remain fully exposed to the air. Counts were made at intervals. The results of the experiment are shown in the table below.

THE EFFECT OF CARBON DIOXIDE ON DIFFERENTIAL MORTALITY

	Jar No. 3		Jar No. 2		Jar No. 1	
Hrs. in CO_2	23.5		46.5		51.5	
Date Removed	Dec. 20, 11:30 A.M.		Dec. 21, 10:30 A.M.		Dec. 21, 3:30 P.M.	
	T. mol.	*T. obs.*	*T. mol.*	*T. obs.*	*T. mol.*	*T. obs.*
Count made Dec. 21, 9 A.M.:						
Alive	66	51				
Dead	10	24				
Count made Dec. 21, 10 A.M.:						
Alive			64[3]	30[3]	48[3]	8[3]
Possibly alive			5	15	21	35
Certainly dead (discolored)			5	31	6	35
Count made Dec. 28:						
Alive	60	38	66	26	59	10
Dead (discolored)	16	37	9	50	16	68
Per cent. dead	21.05	49.33	11.99	65.80	21.33	87.18

SUMMARY

The exposure of larvæ of *Tenebrio molitor* and *Tenebrio obscuris* to elevated temperature for a few hours causes a much greater mortality among the larvæ of *T. molitor* than among those of *T. obscuris*; 37.14 per cent. of *T. molitor* are dead after 3.5 hours at 42° as contrasted with 11.77 per cent. deaths of *T. obscuris*.

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[3] None able to crawl as yet. The feet only are moving.

Exposure to cold for a long period of time causes a differential mortality in the opposite direction, nearly all of *T. molitor* (91 per cent.) remaining alive while 50 per cent. of *T. obscuris* died.

Subjecting a mixture of the larvæ to an atmosphere of pure carbon dioxide for 24 to 51 hours causes a differential mortality in favor of *T. molitor*, only 21.33 per cent. dying after 51.5 hours in the carbon dioxide as contrasted with 87.18 per cent. of *T. obscuris*.

It has been my experience, and I understand that owners of bird stores have noted the same fact, that there is a relatively high death rate among the larvæ of *T. obscuris* under natural conditions, while almost none of the larvæ of *T. molitor* die before pupating.

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